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DEVELOPMENT AND SURVIVAL UNDER OUTDOOR CONDITIONS OF EGGS AND LARVAE OF THE COMMON RUMINANT STOMACH WORM, HAEMONCHUS CONTORTUS[1]

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INTRODUCTION

Pasture rotation has long been one of the measures recommended for the control of roundworm parasites of sheep and other ruminants. This method of control must be based on a knowledge of the length of time that pasture previously occupied by parasitized ruminants should be rested before susceptible animals can be grazed on it without danger of acquiring nematode parasites. To obtain this knowledge, the period of survival of the free-living stages of these nematodes must be known.

Studies that have been made on the free-living stages of the common stomach worm, *Haemonchus contortus*, an important and widespread parasite of sheep and other ruminants, are of two major groups, namely, (1) those concerned with the effects of various environmental factors on the eggs and larvae under laboratory and field conditions and (2) those having to do with the survival of infective larvae on pastures.

REVIEW OF LITERATURE

Ransom (*7*)[2] found that "the eggs and newly hatched embryos possess little powers of resistance, and many die if subjected to freezing or drying," whereas infective larvae exposed outdoors in feces during the winter survived repeated freezing and thawing for 12 weeks and were also resistant to drying for 35 days.

Veglia (*9*) reported the results of both laboratory and field experiments on the survival of the eggs and larvae of *Haemonchus contortus*. This author found that under laboratory conditions temperatures between 68° and 95° F. favored the development of eggs in cultures, whereas at 46° only 30 percent of the eggs had reached the second larval stage after 21 days, and at 32° all the eggs were killed after 48 hours. Exposure of the eggs to 32° or 39° for 24 hours, followed by a return to 75°, killed many of them. Temperatures above 95° became increasingly unfavorable to the development of the eggs, but infective larvae resisted temperatures of 122° for about 2 hours, 108° for a maximum of a month, and 32° for 6 to 7 months. Drying

[1] Received for publication March 16, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 433.

prevented the development of eggs and larvae and killed infective larvae within a few days under laboratory conditions. However, eggs containing embryos resisted drying in air better than did eggs in the early stages of development. Although moisture was necessary for the development of eggs and larvae in cultures, an excess of moisture inhibited their development. Infective larvae, however, survived well in water.

The effects of sunlight and moisture on the survival of eggs and larvae outdoors were also studied by Veglia. Some of the experiments were conducted under artificial conditions; for example, cultures of feces were kept under a glass jar or were watered at regular intervals to reduce drying. From the results of these experiments the following inferences were made. Constantly warm and moist or cloudy weather was favorable to the development of a majority of the eggs, but constantly warm, dry weather, such as occurred during periods of drought, was unfavorable. Constantly dry, cold weather, expecially if the temperature fell to freezing or below for long uninterrupted periods, killed both eggs and larvae. No definite information was available on the effect of variable weather. A high percentage of eggs and freshly hatched larvae were killed if the first 2 days after their deposition on soil were dry and warm, or even when the first day was cloudy, provided the soil was also dry. On the other hand, if the feces were dropped after a heavy rain, numerous eggs survived, especially in grass, even if the first day was sunny and warm. The nature of the pasture and that of the soil itself were factors to be considered. Most of the eggs passed by infected animals failed to reach maturity.

The results of Veglia's experiments were frequently described in general terms, such as "few larvae survived." In other instances the percentages of eggs or larvae surviving or dead were given without the initial or final numbers involved, so that it is difficult to determine whether a sufficiently large number of eggs or larvae was used on which to base conclusions.

Guberlet (*4*) found larvae of *H. contortus* alive after 4 to 5 months in soil and feces cultures. He also found 30 larvae in a pool of water in an infected pasture but gave no further details.

Mönnig (*6*) found that the preinfective stages of *H. contortus* were not resistant to drying, but infective larvae when air-dried indoors survived for a maximum of 4½ months and when exposed to the sun in a mixture of mud and water they survived about 2½ months. Outdoors, the infective larvae survived for as long as 9½ months on grass and clay soil artificially kept moist from below and sheltered from direct sunlight, but survived less than 11 weeks when exposed on black clay soil and grass to dry summer conditions in the Union of South Africa. Data on temperature, humidity, and number of larvae initially used or recovered were not given.

Bozevich (*1*) noted that some larvae exposed on soil to natural weather conditions were alive after 7 months. Eggs refrigerated indoors at −2.2° C. (28° F.) in soil cultures were killed in 6 to 8 hours, depending on the soil type. First-stage larvae in similar cultures were dead after 28 hours, whereas second-stage larvae were dead after a week at 0° C. (32° F.).

Fallis (*3*) found that all eggs of *H. contortus* died after exposure

to −10° C. (14° F.) for 3 to 6 days, whereas the controls, incubated at room temperatures, hatched.

Taylor (*8*) made observations on the longevity of various strongyloid larvae from sheep, rabbits, and horses, the larvae having been placed on grass grown in boxes and kept in an open field. He found that the majority of the larvae recovered from the grass died within the first few weeks of exposure and that only a small proportion remained alive for more than 63 days. Since his experiment with sheep parasites dealt with an unspecified mixture of trichostrongylid larvae, it is not possible to utilize his results in attempts to control any particular species.

Kauzal (*5*), in a brief progress report on work done in Australia, stated that very short periods of desiccation in sunlight were fatal to eggs of both *H. contortus* and *Trichostrongylus* spp., and that some days of continuous rainy or cloudy weather were necessary for the development of a high proportion of larvae of either species. He also reported that the survival period of infective larvae, when exposed to desiccation, is less than previously supposed, and that a rest of 1 to 2 months during periods of continuously dry weather may serve to free a pasture completely of larvae of *H. contortus*.

From the foregoing survey of the literature it is apparent that previous work on the development and survival of eggs and larvae of *H. cortortus* under laboratory or field conditions has been concerned mainly with the discovery of the maximum period of survival under various conditions and, with the exception of Veglia's work, these studies have been primarily qualitative. A quantitative study of the rates of development and survival of the various free-living stages under the influence of natural weather conditions is described in the present paper. This work was carried on at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

MATERIALS AND METHODS

Experimental infections with *Haemonchus contortus* were maintained continuously in young rams that had been raised free of nematodes, except for occasional infections with *Strongyloides papillosus*. These rams were kept indoors in concrete-floored pens that were cleaned daily. Their feces were collected in cloth bags, held firmly against the hindquarters of the sheep by a cloth harness. Each bag was emptied daily into a pail used exclusively for a particular sheep.

For use in an experiment, the pellets passed during the previous 24-hour period were finely ground in a mortar. The entire mass was then thoroughly stirred, and sixteen 50-gm. samples were weighed out. Twelve samples were exposed outdoors as described below and the remaining four samples were used as controls. The control samples were put into separate, covered pint jars and kept at room temperature in a dark closet. A modified Stoll dilution egg count was also made.

The experiments, totaling 30, were performed from August 16, 1939, to October 29, 1940. The first six experiments were begun from 5 days to 2 weeks apart, but after the sixth, they were begun 2 weeks apart except for a gap between experiments 28 and 29. Each experiment was made as follows:

A sandy loam was treated with steam in a closed box to kill any free-living or parasitic nematodes that might be present. Samples of

this heated soil showed no nematodes when examined. Twelve unglazed flowerpots, about 8½ inches high and 8 inches in diameter at the top, were filled with the soil to within 1½ inches of the top and were then planted with seeds of Italian ryegrass (*Lolium multiflorum*) at the rate of about 1.7 gm. to each pot. This grass was raised either in the laboratory or outdoors, depending on the season, to a height of about 3 to 4 inches. The pots were then buried in the ground so that the soil inside and that outside the pots were at about the same level, leaving a rim of about 1½ inches to prevent the feces, eggs, and larvae from washing away. The pots were placed in groups of four in three different locations—full sunlight, partial sun and shade, and full shade. In order to maintain these exposures, the pots were moved to various locations during the year as the sunlight and shade shifted. Those pots exposed to partial sun and shade received sunlight until about noon and shade in the afternoon. After a week in these positions, to permit the soil moisture and temperature to approach those of the surrounding soil, the pots were infected with the twelve 50-gm. samples of feces, which were evenly distributed over the surface of the pots at the base of the grass.

After a given period of exposure outdoors, a pot from each of the three locations was taken into the laboratory and examined to obtain information on the survival of the eggs and larvae. The grass was clipped as close to the soil as possible, placed in a beaker, and left covered with water for 24 hours, after which the washings were examined for the presence of larvae. The feces and the surface layer of soil were scraped up, weighed, and a maximum of 100 gm. was put into the Baermann apparatus. To obtain better sampling of the soil in the pot successive 1-inch layers of soil were removed, weighed, stirred, and a 100-gm. sample from each layer was put into the Baermann apparatus. At least the top 3 inches of soil and in most cases the entire contents of the pot were thus sampled.

The specifications and use of the Baermann apparatus employed in the present experiments have already been given by the writer (*2*). After 48 hours, samples of about 50 to 60 cc. were drawn from the apparatus and examined. When few larvae were present in the sample, all were counted; otherwise they were counted by a combination of subsampling and dilution methods. Since previous experiments indicated that few dead larvae would pass through the Baermann apparatus and be found in the 50- to 60-cc. sample, the dead larvae found in the Baermann samples were considered to have died after going through the apparatus and were added to the number of live larvae found at the time of examination. In most experiments, the live larvae outnumbered the dead ones. For comparison, only the live larvae in the control cultures were counted.

One of the four cultures raised as controls in the laboratory was examined at each interval by a combination of dilution and subsampling methods. The feces and the larvae on the walls and cover of the culture jar were washed into a calibrated beaker and diluted to a known volume of feces and water. After this mixture had stood for several minutes to permit the dried larvae to revive, it was stirred vigorously and a subsample was taken from it. At least three, and in most cases four, subsamples were taken from each suspension. From the number of larvae in these subsamples the total in 50 gm. of feces

was calculated, and the average of the four controls was then used for comparison with the total number recovered from the pots after exposure outdoors.

The presence of viable but still unhatched eggs after a given period of exposure was determined by salt flotations on samples of feces taken from each pot at the time of collection. Moving tadpole or embryo stages within the eggs were the test signs of viability. In addition to these examinations, the hatchability of eggs after exposure late in the fall, in the winter, and early in the spring was determined. In the ninth to twenty-first experiments, 5-gm. samples of feces were taken from each pot after exposure and cultured in the laboratory in Syracuse watch glasses resting in a covered water trap. After an interval of at least a week, the contents of the watch glass and water trap were examined for live larvae.

Air temperatures were measured with a U-type combination maximum-minimum thermometer hung 4 feet above the ground on the north side of a tree trunk. It was read each morning, except on Sundays and holidays, and reset. Measurements of the temperature immediately under the surface of the soil exposed to full sunlight or full shade near the pots were begun in June 1940. Rainfall data were obtained from a station located at the Beltsville Research Center about 1.3 miles from where the experiments were conducted. The gage at this station was read about 9 o'clock each morning.

SURVIVAL OF THE EGGS

VIABILITY OF EGGS AS DETERMINED BY SALT FLOTATIONS

Of the samples from 360 pots examined by the salt flotation method, 44, or 12 percent, in 13 experiments contained viable eggs. These positive experiments were subdivided into 2 groups according to whether they were exposed, during the first period, to mean maximum temperatures above or below 70° F. The low-temperature group included experiments 9, 10, 12, 17, 19, 20, 21, and 30; the high-temperature group, experiments 3, 4, 6, 25, and 28. Further details of these experiments are given in table 3. The results of the salt-flotation examinations are given in table 1, which indicates that unhatched eggs do not remain viable outdoors for more than 13 to 20 days. Within this interval of 20 days, maximum temperatures below 70° favor the longer persistence of viability of eggs than temperatures above 70°.

TABLE 1.—*Viability of eggs of Haemonchus contortus after outdoor exposure as shown by their recovery in salt flotations*

Period of exposure (days)	Total pots examined	Pots containing viable eggs after exposure to mean maximum temperature—		Viable eggs based on total number examined
		Below 70° F.	Above 70° F.	
	Number	*Number*	*Number*	*Percent*
3	66	14	12	39.8
6	90	14	2	17.2
13	90	2	0	2.3
20	90	0	0	0
27	24	0	0	0

HATCHING OF EGGS IN LABORATORY CULTURES AFTER OUTDOOR EXPOSURE

Of a total of 150 laboratory cultures in 13 experiments (Nos. 9 to 21), after outdoor exposure 18 cultures from 6 experiments contained some live larvae. In these 6 experiments the mean maximum air temperatures ranged from 39° to 70° F. during the first 13 days of outdoor exposure. The results of the 18 positive cultures are given in table 2; further details of the experiments, in table 3.

In table 2, the total number of eggs in each pot that could hatch into larvae was computed from the number of larvae hatching in the culture from that pot and added to those for the other two pots to obtain the total number of eggs surviving a given outdoor exposure in an experiment. For comparison with the controls, this total was divided by three times the number of larvae found in parallel control cultures as given in table 3.

The data in table 2 show that eggs do not hatch after exposure to mean maximum temperatures below 70° for more than 6 days. Furthermore, of the 13 experiments that were made, the proportion of eggs that hatched after outdoor exposures of 3 or 6 days, by comparison with the number that hatched in the controls, was very low in 6 experiments and negative in 7.

TABLE 2.—*Effect of previous outdoor exposure on hatching of Haemonchus contortus eggs in 6 experiments*

Experiment No.	Larvae in control cultures	Eggs hatching in laboratory cultures, compared with those in controls, after exposure of—				
		3 days	6 days	13 days	20 days	27 days
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
9	242,250	1.7	0			
10	66,300	.8	0	0	0	
12	308,400		.07	0	0	0
17	324,750		.005	0	0	0
20	159,600	1.1	.3	0	0	
21	105,750	.3	0	0	0	

DEVELOPMENT OF LARVAE

The effect of outdoor exposure in various locations on the development of the eggs, as determined by the percentages of larvae recovered from the pots, is shown in table 3. The larvae recovered after 3 or 6 days' exposure were mostly preinfective, whereas those exposed for longer periods were mostly infective.

The total rainfall for the several periods of exposure was calculated by somewhat different methods. Since Veglia (*9*) concluded that the soil moisture as well as the weather during the early days of exposure affected the survival and development of the eggs, some method of taking this first factor into account was necessary. As no means of measuring soil moisture directly was available, the amount of rainfall on the pots during the 7-day outdoor conditioning period was taken as a rough but practical indicator of the soil moisture at the time of exposure of the eggs. This 7-day total was added to that for the first 3-day period of exposure to obtain the rainfall data pertinent to the 3-day period. The total rainfall during the subsequent exposure period and in the initial 6-day exposures of winter experiments 11 to 18 was found from the amount that fell from the end of the previous period to the end of the period in question.

TABLE 3.—*Percentage of Haemonchus contortus larvae recovered after outdoor exposure of the experimental pots in various locations*

MAXIMUM TEMPERATURES OF 26° TO 65° F. IN FIRST EXPOSURE PERIOD

Experiment No.	Date of infection of soil	Period of exposure	Mean maximum air temperature [1]	Total rainfall [2]	Average larvae in each of 4 controls	Proportion of larvae recovered [3] after exposure in—		
						Shade	Partial sun and shade	Sun
		Days	° *F.*	*Inches*	*Number*	*Percent*	*Percent*	*Percent*
15 [4]	Jan. 31, 1940	6	41	0	6,550	0	0	0
		13	45	.94		0	0	0
		20	44	1.69		0	0	0
10	Nov. 22, 1939	3	47	0	22,100	0	.01	0
		6	48	0		0	0	0
		13	51	.65		0	0	0
		20	52	0		0	0	0
11 [4]	Dec. 6, 1939	6	55	0	26,650	0	0	0
		13	53	.48		0	0	0
		20	51	1.09		0	0	0
19	Mar. 27, 1940	3	63	0	3,400	0	0	0
		6	64	.46		0	0	0
		13	62	2.50		0	0	0
		20	60	.69		0	0	0
13 [4]	Jan. 3, 1940	6	30	.44	30,550	0	0	0
		13	35	.94		0	0	0
		20	32	.85		0	0	0
12 [4]	Dec. 20, 1939	6	42	.60	102,800	0	0	0
		13	39	.20		0	0	0
		20	34	0		0	0	0
14 [4]	Jan. 17, 1940	6	26	.85	2,550	0	0	0
		13	27	.15		0	0	0
		20	33	0		0	0	0
9	Nov. 7, 1939	3	58	.94	80,750	.006	.015	.002
		6	60	0		0	0	.001
		13	60	0		0	0	0
		20	56	0		0	0	0
17 [4]	Feb. 28, 1940	6	47	1.22	108,250	0	0	0
		13	47	0		0	0	0
		20	47	1.92		0	0	0
16 [4]	Feb. 14, 1940	6	42	1.35	450	0	0	0
		13	42	1.36		0	0	0
		20	43	1.22		0	0	0
18 [4]	Mar. 13, 1940	6	48	1.92	36,000	0	0	0
		13	48	0		0	0	0
		20	52	.46		0	0	0
21	Apr. 24, 1940	3	60	2.46	35,250	.003	.006	.01
		6	66	0		0	0	0
		13	70	.62		0	0	0
		20	73	0		0	0	0
20	Apr. 10, 1940	3	64	3.02	53,200	0	0	0
		6	58	.17		0	0	0
		13	57	2.76		0	0	0
		20	60	.02		0	0	0

MAXIMUM TEMPERATURES OF 66° TO 84° F. IN FIRST EXPOSURE PERIOD

7	Oct. 11, 1939	3	70	0	221,200	0.01	0.002	0.0005
		6	68	0		.0005	0	.003
		13	69	.12		0	.03	.06
		20	68	.77		.005	.008	.02
24	June 19, 1940	3	78	0	3,100	0	.03	.3
		6	81	0		0	0	1.8
		13	81	.76		.03	0	0
		20	81	1.50		0	.03	0
5	Sept. 22, 1939	3	83	0	425,100	0	0	0
		6	81	.06		0	0	0
		13	74	3.88		0	.01	0
		20	77	0		0	.02	0
2	Aug. 30, 1939	3	85	0	12,100	0	0	0
		6	86	2.46		0	0	0
		13	84	.53		.1	.03	.08
		20	83	.19		0	0	0
8	Oct. 25, 1939	3	71	.12	215,850	4.7	26.9	2.0
		6	67	.77		.005	1.5	.9
		13	59	1.72		.04	.8	.04
		20	59	0		.02	.04	.02

TABLE 3.—*Percentage of Haemonchus contortus larvae recovered after outdoor exposure of the experimental pots in various locations*—Continued

MAXIMUM TEMPERATURES OF 66° TO 84° F. IN FIRST EXPOSURE PERIOD—Con.

Experiment No.	Date of infection of soil	Period of exposure	Mean maximum air temperature	Total rainfall	Average larvae in each of 4 controls	Proportion of larvae recovered after exposure in—		
						Shade	Partial sun and shade	Sun
		Days	° *F.*	*Inches*	*Number*	*Percent*	*Percent*	*Percent*
28	Sept. 11, 1940	3	70	.36	360, 500	29.3	7.8	7.3
		6	72	.01		1.1	1.2	0
		13	77	0		.5	.3	0
		20	75	.88		.2	.9	0
30	Oct. 9, 1940	3	66	.44	194, 400	2.9	4.6	1.4
		6	70	0		4.4	2.6	.001
		13	63	.47		.01	.004	0
		20	62	.15		0	0	0
22	May 8, 1940	3	73	.62	62, 850	6.1	11.4	.002
		6	76	0		0	0	0
		13	78	.88		.3	3.3	0
		20	76	1.81		.2	0	0
4	Sept. 13, 1939	3	80	.72	483, 900	12.5	15.6	.6
		6	81	0		.3	.9	.3
		13	81	0		.4	.2	.2
		20	78	3.94		.1	.03	0
29	Sept. 25, 1940	3	70	.88	238, 750	26.5	14.5	5.1
		6	68	0		6.4	5.3	.005
		13	69	.84		6.3	8.1	.4
		20	69	.44		1.7	6.9	.1
6	Sept. 27, 1939	3	73	.92	355, 450	34.7	41.2	24.9
		6	71	3.02		24.3	24.5	9.8
		13	73	0		21.7	18.4	8.2
		20	73	0		21.4	18.2	15.1
25	July 3, 1940	3	77	2.26	4, 500	0	.02	0
		6	80	0		1.0	8.9	.4
		13	83	.26		.4	2.6	6.7
		20	86	0		1.3	.1	4.9
3	Sept. 8, 1939	3	84	2.99	287, 000	32.1	26.6	3.0
		6	78	.19		36.1	26.6	5.7
		13	80	0		24.5	20.0	2.8
		20	80	.05		5.4	36.2	1.0

MAXIMUM TEMPERATURES OF 86° TO 93° F. IN FIRST EXPOSURE PERIOD

26	July 17, 1940	3	88	0.26	11, 700	0.2	0	0
		6	91	0		.1	.009	0
		13	93	1.15		9.4	0	0
		20	91	0		.6	0	0
23	June 5, 1940	3	87	.50	10, 450	.6	.9	0
		6	86	0		6.3	.5	0
		13	85	0		12.8	0	.2
		20	84	0		11.9	0	0
27	July 31, 1940	3	86	.71	18, 050	9.6	0	0
		6	86	0		21.4	.1	0
		13	87	0		.1	0	0
		20	85	1.12		4.1	.1	0
1	Aug. 16, 1939	3	93	1.57	24, 500	0	0	0
		6	92	.06		5.4	.05	.004
		13	89	0		10.5	.02	0
		20	88	2.46		1.2	0	0

[1] Mean of maximum air temperatures in shade during entire indicated period of exposure.
[2] For method of computing, see text.
[3] Based on average number of larvae in control cultures.
[4] No larvae recovered from pots exposed 27 days; record omitted.

To facilitate the study of the data in table 3, they are summarized in table 4. The average percentage of recovery was computed by dividing the total of all larvae recovered in a given temperature and rainfall group, exposed for the same period and in the same location, by the total number of larvae in the corresponding controls.

TABLE 4.—*Summary of table 3, showing effect of temperature and rainfall during the early exposure period on the development of larvae*

Location of pots	Period of exposure	Larvae recovered after exposure of experimental pots to mean maximum temperature and rainfall of—				
		26° to 65° F.[1]	66° to 84° F.			86° to 93° F.
		0 to 3.02 inches	0 inch	0.12 to 0.72 inch	0.88 to 2.99 inches	0.26 to 1.57 inches
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Shade	3	0.001	0.003	14.0	32.0	3.0
	6	0	.0002	1.0	23.0	9.0
	13	0	.002	.3	18.0	8.0
	20	0	.002	.09	11.0	4.0
Partial shade	3	.003	.001	14.0	29.0	.1
	6	0	0	1.0	20.0	.1
	13	0	.02	.5	16 0	.006
	20	0	.01	.3	21.0	.04
Sun	3	.001	.001	3.0	12.0	0
	6	.0002	.01	.3	6.0	.002
	13	0	.02	.08	4.0	.03
	20	0	.005	.003	6.0	0
Number of experiments in group		13	4	5	4	4
Total number of larvae in controls for group		508, 500	661, 500	1, 317, 500	885, 700	64. 700

[1] The pots in experiments 11 to 18, inclusive, were first examined after a 6-day exposure.

Of 13 experiments in which pots were exposed to mean maximum temperatures, during the first exposure period, of less than 65°, only 3 yielded a total of 30 preinfective larvae after 3 days and 1 preinfective larva after 6 days. No larvae were recovered after longer exposures. The amount of rainfall varied widely, with no apparent effect on development of the larvae.

The 13 experiments in which the pots were exposed to initial maximum temperatures of 66° to 84° fall into 3 rainfall subdivisions. In the first subdivision, with no rainfall, are 4 experiments in which the numbers and proportions of larvae recovered after all exposures were very low.

In the second rainfall subdivision, containing 5 experiments, the percentages recovered were generally higher than those in the first. Although these percentages were variable, there was no consistent increase in recoveries with increasing rainfall in individual experiments. On the other hand, the degree of exposure to sunlight seemed to affect the recoveries, for the pots exposed to full sunlight yielded lower percentages than those exposed either to full shade or partial sun and shade. After exposure for 6 days there was a sharp drop in the percentages recovered, except in experiment 30, which had a higher recovery at 6 days than at 3 days. The recoveries of infective larvae after 13- and 20-day exposures were below 1 percent of the controls, except for 1 pot.

In the third and highest rainfall subdivision are four experiments which as a group had the highest percentage of both preinfective and infective larvae developing outdoors. Lower percentages were recovered from pots exposed in the sun than from the other two locations, from which recoveries were generally in rather close agreement. The comparatively high average for the 20-day period of exposure in

partial shade was due to the results obtained in experiment 3. The recoveries from experiment 25, much lower than those from the others, cannot be explained on the basis of either temperature or rainfall.

In the high-temperature group, containing four experiments, the recoveries after exposure in partial shade were much lower than those in the middle range of temperatures with some accompanying rainfall, and there was an almost complete absence of larvae in the pots exposed to the sun. An estimate of the temperatures just under the surface of bare soil exposed to the sun during various seasons is provided by the measurements, begun with experiment 25, shown in table 5. The corresponding temperatures taken immediately under the bare soil in the shade approximated closely those in the air in the shade.

TABLE 5.—*Means of maximum temperatures taken immediately under the surface of bare soil in full sunlight and in the air in the shade*

Period	Temperatures taken in the soil exposed to the sun	Temperatures taken in the air in the shade	Difference	Period	Temperatures taken in the soil exposed to the sun	Temperatures taken in the air in the shade	Difference
1940	° *F.*	° *F.*	° *F.*	*1941*	° *F.*	° *F.*	° *F.*
June[1]	121.6	83.6	38.0	January	43.6	39.0	4.6
July	126.7	87.8	38.9	February	50.5	40.0	10.5
August	110.6	81.6	29.0	March	65.5	49.9	15.6
September	97.9	75.8	22.1	April	105.5	73.1	32.4
October	78.3	63.0	15.3	May	126.4	80.8	45.6
November	61.0	52.9	8.1	June	112.9	78.5	34.4
December	53.6	47.6	6.0				

[1] Temperatures for June 26 to 30 only.

As previously stated, Veglia (*9*) found that temperatures above 95° F. become increasingly unfavorable to the development of eggs and that infective larvae resist temperatures of 122° for only 2 hours. Although the pots had a cover of ryegrass, the high temperatures undoubtedly were at least partly responsible for the faster scorching of the grass and the faster drying of the soil and feces observed in the pots exposed in the sun during the hot summer periods and for the resultant low recoveries of larvae from these pots.

DISCUSSION

Before applying the results of the above experiments, it is necessary to determine how closely the field conditons are indicated by the present measurements of weather factors and of their effect on the preparasitic stages of *Haemonchus contortus*.

The maximum air temperatures taken in the shade, used throughout the present experiments, approximate closely those taken immediately below the surface of the soil in the shade but are lower than the soil temperatures in the sun. In addition to this varying exposure to the sun at different seasons, the difference between air temperatures and those on or near the surface of the soil may be affected by the situation and slope of the land, the soil type, and the amount and density of plant cover. In view of these considerations, maximum air temperatures in the shade indicate best the soil-surface temperatures in continuous shade but underestimate those in the sun, where

higher temperatures might favor more rapid development of the eggs in cool weather or destruction in warm weather.

Since the moisture content of the environment of the eggs and larvae could not be measured directly, the amount of rainfall was taken as a measure of the water available for keeping the eggs and larvae moist in their environment. It is recognized that many variable processes and factors may intervene between a given rain and its appearance as moisture in the environment, but for the present, rainfall is taken as an over-all, rough, but practical measure of this factor. As in the case of temperature, local differences in moisture content among pots in different situations may be created by differences in exposure to sun or shade. In addition, differences in rainfall between the site of the experiments and the site of the rain gage, while suspected, could not be measured. However, with all these shortcomings, the present methods of measurement of weather factors have the merit of being easily made under field conditions.

The effect of these weather factors on the eggs and larvae was measured mainly by comparing the number of larvae recovered after outdoor exposure with those developing in the controls. Since the larvae exposed outdoors were recovered in part by means of the Baermann apparatus and then counted, whereas those in the controls were counted directly in water suspension, the comparability of the results of these two methods must be checked.

A study of the effectiveness of the Baermann apparatus in the recovery of infective larvae of *H. contortus* by the writer (*2*) shows that both the number of larvae put into the apparatus and the type of substrate through which they must pass, affect the efficiency of recovery. For example, with a substrate of 100 gm. of the same sandy loam and the same Baermann apparatus used in the present experiments, it was found that an average of 11 percent was recovered when 150 to 420 larvae were put into the apparatus, and 33 percent when 600 to 95,400 larvae were used. With a substrate of 100 gm. of ground sheep feces, these recoveries were 5 and 11 percent, respectively. In addition, the apparatus was shown to perform inconsistently from one experiment to another.

Because of these losses, the number of larvae recovered with the apparatus in the above experiments is not directly comparable with the number in the controls. Correction factors based on the efficiency experiments cannot be applied to the present recoveries with the apparatus because these factors are based on recoveries through homogeneous substrates of soil or feces, whereas in the present experiments 88 percent of all larvae recovered came from the surface layer, which was a variable mixture of soil and feces. However, it is safe to say that the total number of larvae surviving outdoor exposure is higher than the number recovered, especially when the recoveries are low. Consequently the percentages of controls recovered, with which the present paper deals, give a minimum estimate of the proportions of larvae surviving outdoor exposure.

The data on the effect of the early weather conditions on the development and survival of the eggs and larvae of *H. contortus* show that temperature and rainfall exchange places as limiting factors in the development of the eggs at maximum temperatures of about 65° F. When the mean maximum temperature in the shade of the first ex-

posure period is below 65°, few if any eggs hatch no matter how much rain falls, and no infective larvae are recovered after 20 days outdoors. With mean maximum temperatures between 66° and 84°, the development of the eggs into larvae and the survival of the larvae increase with increasing amounts of rainfall during the 7 days before and 3 days after exposure of the eggs. With temperatures above 86°, a range of 0.26 to 1.57 inches of rainfall is accompanied by a moderate degree of survival only in exposures to shade.

The amount of exposure to the sun, when the mean maximum temperatures range above 65°, also affects the degree of development and survival. The lowest recoveries were obtained from the pots exposed in full sunlight. About the same recoveries were obtained from the pots exposed in partial shade as from those exposed in full shade in the middle temperature range. However, in the high temperature range, the recoveries from the pots in partial shade were consistently far lower than those from the pots in full shade, possibly as a result of higher soil temperatures.

Of these two limiting factors, temperature is more reliable than rainfall for the designation of definite periods of the year as unfavorable for the development of the eggs of *H. contortus*, since seasonal trends are more stable and predictable for temperature than for rainfall conditions, in a temperate climate. Further, if the maximum air temperatures are constantly below 65° F., no development of eggs will occur regardless of the amount of rainfall. On the other hand, above 65° only consistently dry periods can be used to designate intervals unfavorable for development.

SUMMARY AND CONCLUSIONS

Development of the eggs and survival of the infective larvae of *Haemonchus contortus* under the influence of natural weather conditions were studied by exposing feces containing eggs of this nematode outdoors in flowerpots under a cover of ryegrass. Twelve pots, examined in groups of 3 at 4 intervals up to 27 days, comprised 1 experiment. The experiments, totaling 30, were conducted from August 1939 to October 1940 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md. The following conclusions are based mainly on a comparison of the number of larvae recovered after exposure outdoors with the number developed in laboratory controls and on the relationship of this ratio with the accompanying outdoor air-shade temperature and rainfall data.

When eggs of *H. contortus* were exposed outdoors for 3 days or more to mean maximum temperatures below 65° F., few preinfective larvae—those recovered after 3 or 6 days' exposure—and no infective larvae—those recovered after 13 or more days—were found during the experiments, regardless of the amount of rainfall.

With mean maximum temperatures between 66° and 84° during the first 3 days, the amount of rainfall in the week preceding and the 3 days following exposure of the eggs considerably affected the degree of their development. After 13 to 20 days outdoors, with no rainfall, infective larvae recovered, based on the number of controls, at no time exceeded an average of 0.01 percent; with 0.12 to 0.72 inch and 0.88 to 2.09 inches of rainfall, the highest averages were 0.5 and

21 percent, respectively. Higher percentages of preinfective larvae were recovered.

With mean maximum temperatures between 86° and 93° and 0.26 to 1.57 inches of rainfall, the highest recovery of infective larvae was 8 percent, after 13 days.

Exposure to full sunlight resulted in lower yields of larvae than exposure to full shade or partial shade. In the middle temperature range, recoveries from exposure to full and partial shade were about the same. In the upper temperature range recoveries from the former were consistently higher than from the latter.

Recoveries from the pots provide only a minimum estimate of the number of larvae surviving outdoor exposure because of the losses in the Baermann apparatus.

Eggs do not survive exposure to mean maximum temperatures below 70° for more than 6 to 13 days, as shown by cultures, or for more than 13 to 20 days, as shown by salt flotations. Above 70°, salt flotations indicate that eggs do not survive more than 6 to 13 days outdoors.

In designating large periods of the year as unfavorable for the survival and development of the eggs of *H. contortus*, temperature is a more reliable weather factor than rainfall.

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MEIOTIC STUDIES OF CROSSES BETWEEN FRAGARIA OVALIS AND × F. ANANASSA[1]

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INTRODUCTION

The value of the native Rocky Mountain strawberry (*Fragaria ovalis* (Lehm.) Rydb.) for hybridizing with cultivated varieties (× *F. ananassa* Duch.) to develop new varieties having greater winter hardiness has long been recognized (*2, 8, 10*).[3] However, the karyological investigations of Longley (*17*), East (*4, 5*), Mangelsdorf and East (*18*), Kihara (*13*), Yarnell (*29, 30*), Ichijima (*11*), Fedorova (*6*), Lilienfeld (*15, 16*), Kuz'min (*14*), Rozanova (*25*), and Dogadkina (*3*) show the importance of meiotic studies as an aid to any breeding program involving wide crosses between polyploid species and forms within the genus *Fragaria*. The researches of these investigators emphasize particularly the part that asynapsis, conjugation of more than two chromosomes to form multivalents during meiosis, and type of chromosome conjugation (autosyndesis, allosyndesis, or a combination of the two) may play in the breeding of improved varieties.

The importance of asynapsis and of the conjugation of more than two chromosomes to form multivalents during meiosis lies in the effect that they have upon fruitfulness. By adversely affecting fruitfulness asynapsis of the chromosomes during meiosis, if of frequent occurrence, may be one of the major factors contributing to the failure of a breeding program. Likewise, if homology exists between different genoms coming from the same polyploid species, as well as between genoms coming from different polyploid species, conjugation of more than two chromosomes to form multivalents might be of frequent occurrence. If such were the case, one would expect fruitfulness to be reduced materially, possibly to the extent that the accomplishment of the objectives of the breeding program would be threatened.

Turning to the type of conjugation of the chromosomes, if autosyndesis (the pairing, in a polyploid, of chromosomes derived from the same parent) was occurring, then variation in the F_2 population would be limited largely to that taking place within the F_1 hybrid, and the chances for recombining any of the desirable characters of both parents into a single segregate would be decidedly reduced. On the other hand, allosyndesis (pairing in a polyploid of chromosomes derived from opposite parents) would allow for the maximum segregation of the genes differentiating the interspecific characters. A combination of autosyndesis and allosyndesis would result in an

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[3] Italic numbers in parentheses refer to Literature Cited, p. 447.

intermediate condition as regards the possibilities for maximum segregation of these same genes. Consequently, information concerning the type of conjugation of the chromosomes during meiosis of the F_1 hybrids would be of considerable value in planning and conducting a breeding program.

The objectives of the studies herein reported were (1) to determine whether asynapsis and the association of more than two chromosomes to form multivalents during meiosis occur frequently enough to affect a breeding program adversely and (2) to ascertain the type of conjugation that occurs during meiosis of the F_1 plants.

REVIEW OF LITERATURE

With the exception of a few varieties produced by Georgeson (*8*), the cultivated strawberries have descended from crosses involving *Fragaria virginiana* and *F. chiloensis*, both of which are octoploids. The basic number of chromosomes in *Fragaria* is seven; hence, the chromosomes of the cultivated strawberry are comprised of four genoms each having seven pairs of chromosomes. The best available information as to the relation between these four sets of chromosomes and between the chromosomes of different species is that obtained from the genetic and karyological investigations of crosses between species of different chromosome numbers and, to a more limited extent, between species of the same chromosome number.

The investigations of Longley (*17*), East (*4, 5*), Mangelsdorf and East (*18*), Kihara (*13*), and Yarnell (*29, 30*) showed that at least some chromosomes of the four genoms of seven pairs of chromosomes are homologous. Yarnell (*29, 30*), from cytological and genetic studies, came to the conclusion that the four sets of chromosomes of the octoploid had a common origin and that corresponding chromosomes of each set were originally homologous. After examining microsporocytes from diploid-tetraploid and diploid-octoploid crosses, Yarnell reported not only pairing between chromosomes from both parents but also autosyndesis among the remaining genoms of the tetraploid parent and the remaining sets of the octoploid parent. He found that in many cases the counts at first metaphase indicated complete pairing, involving the nonhomologous chromosomes of the extra genom, and, in addition, he found secondary association taking place between disomes.

The findings of Ichijima (*11*), Fedorova (*6*), Lilienfeld (*15, 16*), Kuz'min (*14*), Rozanova (*25*), and Dogadkina (*3*) in regard to the homologous relations between the chromosomes of the octoploid forms are well summarized by the following statement from Rozanova (*25*):

> . . . it may be deduced that the evolution of species of *Fragaria* has proceeded in the direction of autopolyploidy or close allopolyploidy. From this it follows that the hypothesis as to the origin of cultivated varieties from a cross between *F. virginiana* and *F. chiloensis* needs supplementing to the extent of stating that *F. virginiana* and *F. chiloensis* are also probably autopolyploids or close allopolyploids with homologous genoms.

From the above investigations it is evident that the haploid chromosome complement of the octoploid forms of *Fragaria* may be symbolized as follows:

F. ovalis—Ao_1, Ao_2, Ao_3, Ao_4
F. chiloensis—Ach_1, Ach_2, Ach_3, Ach_4

F. virginiana—Av_1, Av_2, Av_3, Av_4
× *F. ananassa*—Acv_1, Acv_2, Acv_3, Acv_4

These formulas are closely patterned after those given by Rozanova (*25*). The *A* is used to show that all the chromosomes of one species are homologous with the chromosomes of any other and that a certain amount of homology exists between genoms within a species. *Acv* indicates that the genoms of × *F. ananassa* through hybridization descended from those of *F. chiloensis* and *F. virginiana*.

From the studies of the various workers cited, it can be seen that there are a number of possibilities as regards chromosome behavior during meiosis in the F_1 hybrids between *Fragaria ovalis* and × *F. ananassa*. Asynapsis may or may not be of frequent occurrence. Associations of more than two chromosomes to form multivalents may be the rule, or such associations may occur occasionally or only very infrequently. Finally, the four genoms from *F. ovalis* may pair during meiosis with the four genoms from the cultivated varieties (allosyndesis); two genoms from *F. ovalis* may pair with two other genoms from that species and hence two genoms of × *F. ananassa* with two other genoms of that species (autosyndesis); and both allosyndesis and autosyndesis may occur either at random or in disproportionate frequencies.

EXPERIMENTAL MATERIAL AND DESIGN

The parents and F_1 hybrids were used in the studies. The design of the experiment was that of a randomized complete block and the variates were as follows:

Group I (collections of *Fragaria ovalis*): A, 37501; B, 361477; C, 36979.
Group II (F_1 hybrids): A, Dorsett × 37501; B, Gem × 361477; C, Fairfax × 36979.
Group III (varieties of × *F. ananassa*): A, Dorsett; B, Gem; C, Fairfax.

Each group hereafter will be called a species containing three variates. Although group II cannot strictly be classed as a species, it is clear that any differences between this group and either of the other groups must be due to genetic differences between groups I and III. Hence, from genetic considerations one is justified in considering the genetic variation between these three groups as due to inherent differences between species. It follows that, if the differences between the totals of these three groups can be attributed to chance deviations, it cannot be concluded, so far as these collections and varieties are concerned, that there are any differences between species.

The grouping on the basis of A, B, and C is as follows:

Group A: I, *Fragaria ovalis* (37501); II, F_1 (Dorsett × 37501); III, × *F. ananassa* (Dorsett).
Group B: I, *F. ovalis* (361477); II, F_1 (Gem × 361477); III, × *F. ananassa* (Gem).
Group C: I, *F. ovalis* (36979); II, F_1 (Fairfax × 36979); III, × *F. ananassa* (Fairfax).

Again, each group, which hereafter will be designated as a strain, contains three variates. The totals for groups A, B, and C will show statistically significant differences only if there are genotypic differences between the variates of group I, group III, or both I and III. Hence, one is justified in attributing any differences between A, B, and C to differences between strains.

The measure of asynapsis used in these studies was (1) the number of pollen mother cells in 100 observations showing some univalent chromosomes during metaphase I, (2) the number of cells in 100 observations showing at least 1 lagging chromosome during early telophase I, (3) the number of cells in 100 observations showing some chromosomes not on either equatorial plane during metaphase II, and (4) the number of cells in 100 observations showing some lagging chromosomes during early telophase II in any member of the potential tetrads. Thus, 4 different phases of meiosis were studied. In addition, the previously mentioned 100 pollen mother cells in metaphase I were examined to obtain some estimate of the frequency of occurrence of associations of more than 2 chromosomes during meiosis. Four plants of each variate were included in the studies, and 25 cells of each plant were examined. The 4 plants were from 4 different replicates.

With the present limited knowledge of the morphology of the strawberry chromosomes, it is not possible to determine through cytological examination of the stages of division whether autosyndesis, allosyndesis, or a combination of the two is occurring during meiosis. However, indirect but reliable evidence as to the type of conjugation that is occurring can be obtained by studying the means of certain characters of the parents and the hybrid populations.

The young anthers were killed in a solution of 3 parts of absolute alcohol to 1 part of glacial acetic acid and stored in 70-percent alcohol. Belling's (*1*) iron-acetocarmine method was employed in staining the material.

At this time it should be noted that the plants of any particular collection may not be alike genetically. For example, collection 37501 is composed of at least two strains, one of which has pistillate flowers while the other has perfect flowers. The strain possessing the perfect flowers was used in the cytological studies.

ANALYSIS OF THE DATA

Since the data were enumeration data, the method of analysis chosen was to partition χ^2 into its components (see Fisher, 7) and calculate the heterogeneity χ^2 when it seemed desirable. However, the formulas developed and used in calculating the different χ^2's are not available elsewhere. Therefore, since they are of general application (particularly for enumeration data such as those obtained in germination studies), and since they materially simplify the calculations, detailed illustrations of their application are given.

The components of χ^2 together with their corresponding degrees of freedom are as follows:

Variation due to:	*Degrees of freedom*	Variation due to—Continued.	*Degrees of freedom*
Main effects	7	Interactions	28
Species	2	Species × strains	4
Strains	2	Species × phases	6
Phases	3	Strains × phases	6
		Species × strains × phases	12
		Total	35

The relation between these components and the division used in an analysis of variance is readily recognized.

The formula employed in obtaining χ^2 for the main effects, interactions, and total is as follows:

$$\chi^2=\left[\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx\right]+\frac{Sx}{Sy}\left[\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx\right]$$

in which

N= total number of items classified
Nu= number of items classified for the lowest category of the table
x= number of items in any one category showing one or the other of the alternative phenomena
y= number of items in any one category showing the other alternative phenomenon.

The detailed calculations for obtaining the total χ^2 for the data given in table 1 are given in table 2.

TABLE 1—*Univalent and lagging chromosomes in 100 cells of collections of Fragaria ovalis, varieties of × F. ananassa, and their F_1 hybrids during meiosis*

DETAILED DATA NOT GROUPED

Variate	First division (I)		Second division (II)		Total
	Metaphase	Telophase	Metaphase	Telophase	
Fragaria ovalis:	*Number*	*Number*	*Number*	*Number*	*Number*
37501	4	5	2	4	15
361477	6	4	3	1	14
36979	8	3	3	4	18
Total	18	12	8	9	47
F_1 hybrid:					
Dorsett × 37501	3	0	2	0	5
Gem × 361477	1	2	1	0	4
Fairfax × 36979	5	4	4	5	18
Total	9	6	7	5	27
× *F. ananassa:*					
Dorsett	2	1	0	1	4
Gem	5	2	2	1	10
Fairfax	1	0	3	2	6
Total	8	3	5	4	20

DATA GROUPED FOR SPECIES AND STRAINS

Species group	Row symbol	Sets [1] involving—			Total
		37501 and Dorsett	361477 and Gem	36979 and Fairfax	
Fragaria ovalis	r_1	15	14	18	47
F_1 hybrids	r_2	5	4	18	27
× *F. ananassa*	r_3	4	10	6	20
Total		24	28	42	94

[1] Column symboi for column 3=*a*, for column 4=*b*, and for column 5=*c*.

To complete the illustration of the method of partitioning total χ^2 into its components, the data grouped on the basis of one of the interactions need to be considered (see table 1). The calculations are given in table 2. The χ^2 for the data based on species and strains

is 27.549. But this χ^2 for species and strains is composed of the χ^2 due to differences between species, the χ^2 due to differences between strains, and the χ^2 due to the interaction between species and strains. The χ^2 values for species and strains, also given in table 2, are 12.868 and 5.855. Subtracting these two values from the χ^2 value 27.549 gives the χ^2 (8.826) attributable to the interaction between species and strains.

TABLE 2.—*Details for calculating total and partitioned χ^2's for data in table 1*

Formula	Total χ^2	χ^2 for species and strains	χ^2 for species	χ^2 for strains
N	3,600	3,600	3,600	3,600
Nu	100	400	1,200	1,200
Sx	94	94	94	94
Sy	3,506	3,506	3,506	3,506
Sx^2	376	1,262	3,338	3,124
$Sx.Nu$	9,400	37,600	112,800	112,800
$\frac{N}{Sx.Nu}$	0.382978723	0.095744681	0.031914894	0.031914894
$\frac{N}{Sx.Nu}Sx^2$	144.000000	120.829787	106.531916	99.702129
$\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx$	50.000000	26.829787	12.531916	5.702129
$\frac{Sx}{Sy}$	.026811181	.026811181	.026811181	.026811181
$\frac{Sx}{Sy}\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx$	1.340559	.719338	.335995	.152881
$\left[\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx\right]$ $+\frac{Sx}{Sy}\left[\left(\frac{N}{Sx.Nu}Sx^2\right)-Sx\right]$	51.340559	27.549125	12.867911	5.855010
χ^2	51.341	27.549	12.868	5.855

Tables similar to the lower part of table 1 can be compiled for species and phases and for strains and phases. The methods of calculating the different χ^2's for the two resulting tables are identical with those used in calculating the various χ^2's for species and strains, and hence need not be given here. The χ^2 for the second-order interaction (species×strains×phases) may be obtained by subtracting the χ^2's for main effects and the χ^2's for the first-order interactions from the total χ^2, which, as previously calculated, is 51.341. All of these χ^2's are listed under Experimental Results (see table 4).

From table 2 and a probability table for χ^2, it can be seen that the odds are rather great against deviations as large as those noted between species occurring by chance. The same, to a lesser extent, is true of the differences between strains. This means that the interaction χ^2 (8.826) is not suitable for testing whether the deviations between the frequency distributions of table 1 for species in respect to strains can be attributed to chance (see Mather, *19*). Hence, it is necessary to calculate a heterogeneity χ^2.

The formula employed in calculating the heterogeneity χ^2 i sas follows:

$$\chi^2 = W + W'$$

in which

$$W = \frac{Sx}{Sa}\left(\frac{a_1^2}{Sr_1}+\frac{a_2^2}{Sr_2}+\ \ldots\ +\frac{a_n^2}{Sr_n}\right)+\frac{Sx}{Sb}\left(\frac{b_1^2}{Sr_1}+\frac{b_2^2}{Sr_2}+\ \ldots\ +\frac{b_n^2}{Sr_n}\right)$$
$$+\ \ldots\ +\frac{Sx}{Sz}\left(\frac{z_1^2}{Sr_1}+\frac{z_2^2}{Sr_2}+\ \ldots\ +\frac{z_n^2}{Sr_n}\right)-Sx$$

W' is obtained by substituting the values of y for those of x in table 1 and solving the formula just given for W. For example, in substituting y for x, cell r_1, a_1 becomes 385 (400−15) instead of 15; r_2, a_2 becomes 395 (400−5); etc. Explanations of other symbols follow:

Sx=total number of the items showing one or the other of the alternative phenomena
Sy=total number of the items showing the alternative phenomenon
$Sa, Sb, \ldots, Sz$=total number of items in the designated column
$a_1, a_2, \ldots, a_n$=number of items in the designated category of column a
$b_1, b_2, \ldots, b_n$=number of items in the designated category of column b
$z_1, z_2, \ldots, z_n$=number of items in the designated category of the last column
$Sr_1, Sr_2, \ldots, Sr_n$=total number of items in the designated row.

From the above formula it will be noted that $a_1^2, b_1^2, \ldots, z_1^2$ are divided by Sr_1. Hence, machine calculation can be much simplified by expressing Sr_1 as a decimal fraction and locking the common multiplier in the machine. The same is true for the other calculations involving Sr. The detailed calculations for the lower part of table 1 follow:

$$Sx=94;\ Sa=24;\ Sb=28;\ Sc=42$$

$$S\left(\frac{a^2}{Sr}\right)=6.513160;\ S\left(\frac{b^2}{Sr}\right)=9.762806;\ S\left(\frac{c^2}{Sr}\right)=20.693617$$

$$\frac{Sx}{Sa}=3.916667;\ \frac{Sx}{Sb}=3.357143;\ \frac{Sx}{Sc}=2.238095$$

$$W=10.599295;\ W'=0.230365;\ \chi^2=10.829660$$

In such problems as those illustrated in which the main effects differ materially, the heterogeneity χ^2 is preferred to the interaction χ^2 obtained by partitioning the total χ^2 into its components. However, in many problems it is not necessary to calculate the heterogeneity χ^2, which involves much more work.

EXPERIMENTAL RESULTS

It can be seen from table 1, without detailed statistical analysis, that meiosis is essentially normal as regards synapsis. This fact is readily appreciated when it is recalled that the figures in the individual categories represent the number of cells among 100 examined that showed at least 1 univalent chromosome. Hence, asynapsis is not frequent enough to interfere materially with the obtaining of double crosses and advanced and backcross populations. It follows, then, that asynapsis is not a factor in breeding new varieties of strawberries by the hybridization method in which × *Fragaria ananassa* and *F. ovalis* are used as parents.

The occurrence of associations of more than two chromosomes to form multivalents during meiosis was so infrequent as not to merit tabulation. It may be concluded, therefore, that this phenomenon also presents no problem in breeding strawberries by the hybridization method in which × *Fragaria ananassa* and *F. ovalis* are used as the parents.

The third major problem is that of determining the type of conjugation occurring during meiosis of the F_1 hybrids. If autosyndesis is taking place during meiosis of the F_1 hybrids between × *Fragaria ananassa* and *F. ovalis*, then the × *F. ananassa* chromosomes are pairing with × *F. ananassa* chromosomes and the *F. ovalis* chromosomes with *F. ovalis* chromosomes. By using the previously given symbols for the chromosome complements of × *F. ananassa* and *F. ovalis*, autosyndesis in the F_1 hybrids may be illustrated as follows:

× *F. ananassa:*
Acv$_1$; *Acv*$_2$; *Acv*$_3$; *Acv*$_4$
Acv$_1$; *Acv*$_2$; *Acv*$_3$; *Acv*$_4$
F. ovalis:
Ao$_1$; *Ao*$_2$; *Ao*$_3$; *Ao*$_4$
Ao$_1$; *Ao*$_2$; *Ao*$_3$; *Ao*$_4$
F_1 hybrid:
Acv$_1$; *Acv*$_2$; *Ao*$_1$; *Ao*$_2$
Acv$_3$; *Acv*$_4$; *Ao*$_3$; *Ao*$_4$

From the above it is clear that, if autosyndesis is occurring, all of the F_2 populations would be composed of four complete sets of × *F. ananassa* chromosomes and four complete sets of *F. ovalis* chromosomes. Hence, if × *F. ananassa* chromosomes do not conjugate with *F. ovalis* chromosomes, there is no opportunity for segregation of the genes differentiating the interspecific characters, that is, those genes differentiating the characters by which the species differ.

Similarly, allosyndesis in an F_1 hybrid may be illustrated as follows:

Acv$_1$; *Acv*$_2$; *Acv*$_3$; *Acv*$_4$
Ao$_1$; *Ao*$_2$; *Ao*$_3$; *Ao*$_4$

From this illustration it can be seen that the maximum opportunity for segregation of the genes differentiating the interspecific characters would occur during meiosis.

One form of a combination of the two types of conjugation in an F_1 hybrid may be illustrated as follows:

Acv$_1$; *Ao*$_1$; *Acv*$_3$; *Acv*$_4$
Acv$_2$; *Ao*$_2$; *Ao*$_3$; *Ao*$_4$

From this illustration it can be seen that in some cases × *Fragaria ananassa* chromosomes are paired with × *F. ananassa* chromosomes; in other cases they are paired with *F. ovalis* chromosomes. It follows that in some instances *F. ovalis* chromosomes are paired with *F. ovalis* chromosomes; in others they are paired with × *F. ananassa* chromosomes. Hence, the opportunity for segregation of the genes differentiating the interspecific characters is intermediate between those of autosyndesis and allosyndesis.

From these considerations it is evident that the type of pairing of the chromosomes (autosyndesis, allosyndesis, or a combination of the two) materially influences the segregation of the genes that differentiate the interspecific characters. The commonest method of chromosome pairing of polyploid organisms is allosyndesis. With this fact in mind, Wright (*28, p. 45*) has shown that—

> a random-bred stock derived from n inbred families will have $\frac{1}{n}$th less superiority over its inbred ancestry than the first cross or a random-bred stock from which the inbred families might have been derived without selection.

Kiesselbach (*12*), working with plants, reached a similar conclusion.

Wright has developed formulas for use when the effects of the genes differentiating the characters under consideration are geometrically cumulative. For these formulas and their application, the reader is referred to Powers (*22*) and Powers and Lyon (*23*). These formulas as well as those based on the assumption that the effects of the genes are arithmetically cumulative assume that allosyndesis is occurring during meiosis of the F_1 hybrid. Thus it is clear that, if the means of the interspecific characters can be predicted by the use of Wright's formulas, allosyndesis must be occurring during meiosis of the microsporocytes and the megasporocytes at least as regards the chromosomes containing the genes which differentiate the characters under consideration. On the other hand, if autosyndesis is the rule, segregation of the genes differentiating the interspecific characters would be materially limited and the means of the characters for the F_2 populations should closely approximate the means of the same characters for the F_1 populations.

In making these tests the studies will be more conclusive if the magnitude of the effects of the genes differentiating the two species is quite large as regards the character under consideration and if the characters are based on absolute rather than observational measurements. The characters which meet these specifications are plant height measured in centimeters, number of days from May 1 to first bloom, and number of days from first bloom to first fruit ripe. Although based only on observational grades, the degree of winter injury was very marked and, therefore, this character also was included in the study. The degree of winter injury increases from grade 1 to grade 5.

The data are presented in table 3. The theoretical means listed are those calculated on the assumption that the effects of the genes differentiating the respective characters are arithmetically cumulative. The fit between the theoretical means calculated on the assumption that the effects of the genes are geometrically cumulative and the obtained means (with the exception of the means for grades of winter injury) was definitely poorer than the fit between the means calculated on the assumption that the effects of the genes are arithmetically cumulative and the obtained means. Since such is the case, there would not be much point in listing the theoretical geometric means. In respect to winter injury the geometric mean for the backcross to Fairfax fitted the obtained mean better than did the arithmetic mean, but for the F_2 population the reverse was true.

TABLE 3.—*The obtained and theoretical arithmetic means and their standard errors for different characters of the cross Fairfax × Fragaria ovalis (36979)*

Variate	Winter injury		Plant height		Period from May 1 to first bloom		Period from first bloom to first fruit ripe	
	Obtained	Theoretical	Obtained	Theoretical	Obtained	Theoretical	Obtained	Theoretical
Fragaria ovalis (36979):	*Grade*	*Grade*	*Centimeters*	*Centimeters*	*Days*	*Days*	*Days*	*Days*
Asexual	1.00±0.170		11.3±0.597		14.2±0.696		46.0±0.943	
Selfed (S_1)	1.06± .124		8.8± .554		14.5± .477		49.1± .605	
Hybrid populations:								
Backcross [(Fairfax×36979)×36979]	1.07± .141	1.16±0.112	16.3± .633	14.5±0.448	14.3± .517	15.3±0.371	44.6± .702	43.8±0.510
F_1 (Fairfax×36979)	1.32± .146		17.7± .668		16.3± .260		40.8± .389	
F_2 (Fairfax×36979)	2.03± .107	2.19± .111	13.1± .345	14.1± .475	22.3± .472	19.7± .870	40.5± .330	40.2± .591
Backcross [Fairfax×(Fairfax×36979)]	2.79± .132	3.21± .110	14.2± .395	13.7± .501	25.2± .610	24.1±1.173	36.3± .515	36.9± .662
×*F. ananassa* (Fairfax):								
Selfed (S_1)	4.65± .169		9.6± .748		31.8±2.332		33.0±1.265	
Asexual	5.10± .165							

The means for winter injury and for days from May 1 to first bloom ranged from that of *Fragaria ovalis* asexually propagated to that of Fairfax asexually propagated or self-pollinated; for days from first bloom to first fruit ripe the means ranged from that of the progeny of Fairfax self-pollinated to that of the progeny of *F. ovalis* self-pollinated. In plant height, for which the F_1 hybrid shows decided heterosis, the means ranged from that of the progeny obtained by self-pollinating *F. ovalis* to that of the F_1 hybrid. These data are in accord with what would be expected if allosyndesis was occurring during meiosis of the F_1 hybrid. It will be recalled that in case autosyndesis was the common mode of behavior the mean of the F_2 population would be expected to be similar in magnitude to that of the F_1 hybrid. As may be seen from the data in table 3, such definitely was not the case as regards winter injury, plant height, and days from May 1 to first bloom. As regards days from first bloom to first fruit ripe, a comparison of the means for the F_1 hybrid and the F_2 population does not indicate whether allosyndesis or autosyndesis is the rule. The reason for this is that the theoretical arithmetic mean based on allosyndesis is so close to that of the F_1 hybrid that any differences noted can logically be attributed to chance deviations. Probably the most convincing evidence that allosyndesis rather than autosyndesis or a combination of allosyndesis and autosyndesis is the rule during meiosis of the F_1 hybrid is that in 10 cases out of a possible 12 (see Tippett *27, p. 54*) the differences between the obtained and theoretical means can logically be attributed to chance deviations.

In summing up it may be said that the evidence, which is rather conclusive, is preponderantly in favor of allosyndesis as the type of conjugation that occurs during meiosis of the F_1 hybrids, and it appears certain that any deviation from allosyndesis is not of sufficient importance to interfere materially with obtaining the objectives of a breeding program in which $\times$ *Fragaria ananassa* (variety Fairfax) and *F. ovalis* (collection 36979) are the parents.

From the foregoing it can be seen that the major problems which the experiment was designed to answer have been solved. However, the data furnish additional information which, though of only minor importance to the breeding program at Cheyenne, may be of interest to other investigators working with the cytogenetics of *Fragaria*.

The χ^2 values for testing goodness of fit between the theoretical based on the supposition that there are no differences between species, between strains, or between phases, together with those χ^2 values for testing whether the interactions are statistically significant, are given in table 4. From these data it seems probable that differences exist between species, between strains, and between phases. However, the differences between strains are not so well established statistically as the differences between species or between phases. The only interaction approaching statistical significance is that between species and strains. The heterogeneity χ^2 for this interaction is 10.830, which is highly significant.

The meaning of these differences can best be found by examining the data in table 1. The differences between species there shown were due to the fact that failure of chromosome pairing was somewhat greater in the native Rocky Mountain strawberry than in the cultivated strawberry. The possible differences between strains were due to the fact that the failure of chromosome pairing was at least

partially dominant in the F_1 hybrid of Fairfax × 36979 and at least partially recessive in the F_1 hybrids of Dorsett × 37501 and Gem × 361477. The differences between phases were due to the fact that the number of cells showing irregularities was greater for the metaphase of the first division than for the telophase of that division and either stage of the second division. The explanation for this may be the same as that found by Powers (*21*) in similar studies with *Triticum aestivum*.

TABLE 4.—χ^2 *values for the different components*

Source of variation	Degrees of freedom	χ^2
Main effects:		
Species	2	12.868
Strains	2	5.855
Phases	3	7.909
Interactions:		
Species × strains	4	8.826
Species × phases	6	3.037
Strains × phases	6	4.020
Species × strains × phases	12	8.826
Total	35	51.341

Environment is a factor that must be considered in evaluating the importance of the frequency of failure of chromosome pairing to a crop-improvement program. The investigations of Stow (*26*), Heilborn (*9*), Randolph (*24*), and Myers and Powers (*20*) definitely show that the differences in environment encountered under field conditions have diverse effects on synapsis. So it must be concluded that, even though asynapsis influences the breeding program very little under the environmental conditions existing at Cheyenne, Wyo., it may be of major importance under the environmental conditions of other locations. For this reason, selections made at Cheyenne from material resulting from crossing ×*Fragaria ananassa* and *F. ovalis* may fail when grown in other localities.

SUMMARY

Cytological data, taken from three cultivated varieties of strawberry (×*Fragaria ananassa*), three collections of the native Rocky Mountain strawberry (*F. ovalis*), and three F_1 interspecific hybrids involving these collections and varieties, are reported.

New formulas for χ^2 are given, and their application is illustrated. These formulas reduce the labor involved in calculating χ^2 when the number of categories of the table used in the calculations is large.

The breeding data show rather conclusively that meiotic instability is at most a minor problem under the environmental conditions prevailing at Cheyenne, Wyo.

The segregation of the genes differentiating certain characters, as indicated by the means of these characters for different populations, furnishes rather convincing evidence that allosyndesis is the rule during meiosis of the F_1 hybrids.

So far as meiotic irregularities and the method of pairing of the chromosomes are concerned, there does not seem to be any reason why the economically important characters of ×*Fragaria ananassa*

and *F. ovalis* cannot be recombined into a single variety adapted to production under the environmental conditions encountered at Cheyenne, Wyo.

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